FURTHER OBSERVATIONS ON SUPERNUMERARY CHROMOSOMES, AND SEX RATIOS IN DIABROTICA SOROR.

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SUPERNUMERARY CHROMOSOMES.

In the summer of 1910 while I was enjoying the privileges and hospitality of the Marine Biological Laboratory at La Jolla, California, I took advantage of the opportunity to study the male germ cells of *Diabrotica soror* from a new locality. Having previously ('08) found supernumerary chromosomes varying in number from one to five in about 50 per cent. of the male individuals of *Diabrotica soror* at Mountain View, California, and *Diabrotica 12-punctata* at Bryn Mawr, Pa., I was interested to see whether supernumeraries would be found in the same proportion in a third locality.

Testes from a hundred individuals were studied in aceto-carmine preparations. The greater part of the material was collected in a corn-field in the open country between La Jolla and the new laboratory which is two miles north of the town. A few were obtained from a rose-garden in La Jolla and one lot of 68 males and females was collected for me by Miss Myrtle Johnson on corn in a garden at National City, just south of San Diego. Individual records were kept for each lot, but the conditions with respect to number of supernumeraries proved to be about the same for the three collecting grounds.

To my surprise I found supernumeraries scarce. In the first 25 males examined, 21 had no supernumeraries and 4 one; while out of the first 25 examined the same summer at Mountain View 15 had no supernumerary, 7 one and 3 two; and in the first 25 at Mountain View in 1909, there were 13 with no supernumerary, 9 with one, 2 with two and 1 with three. In the La Jolla material the 89th individual was reached before a case of two supernumeraries was met with, and in the first 100 males 79 had no supernumerary, 20 one, and 1 two. The follow-

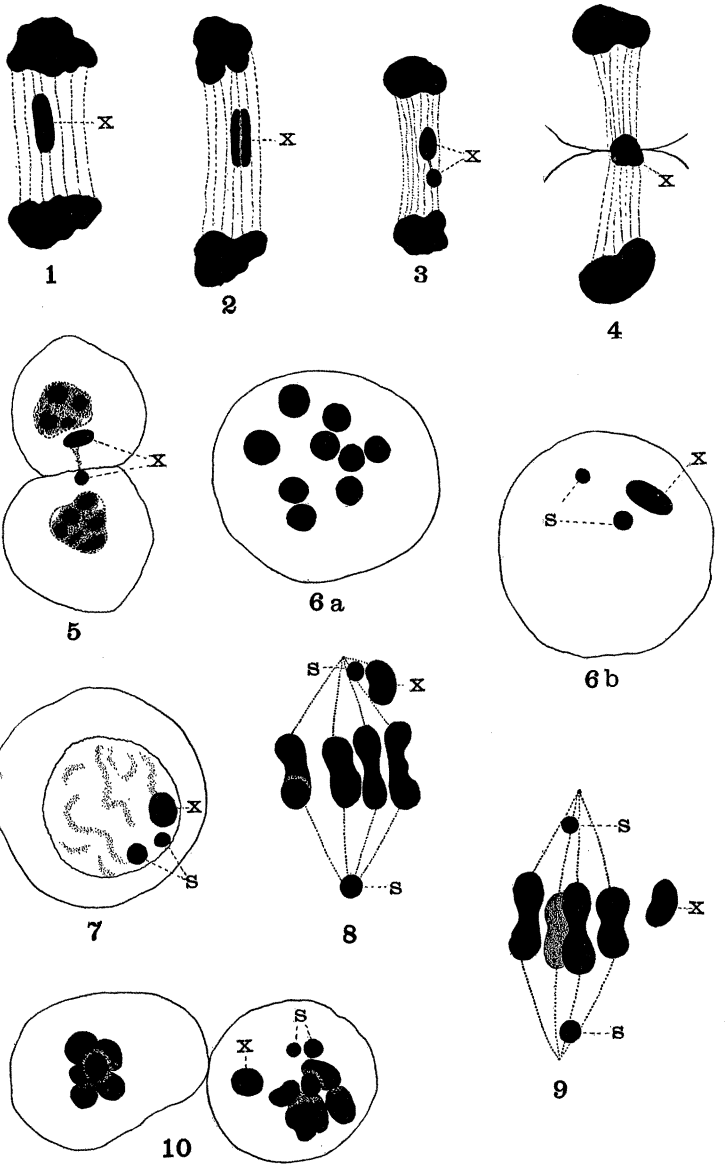
ing table shows the per cent. of supernumeraries in the two species different years and in different localities.

Number of Supernumeraries.	0	1	2	3	4	5
D. sor., Mt. V., '07, June 23–Aug. 7.	51	35	11	2	1	
D. 12–p., B-M., '07, Oct. 3–9.....	48	33	15	3	1	
D. s., Mt. V., '09, July 10–Aug. 12..	43	44	10	3		
D. s., Mt. V., '09, Aug. 21–Sept. 15.	46	38	10	4		2
D. s., Mt. V., '10, July 28–Sept. 1...	52	29	16	3		
D. s., La J., '10, June 17–July 4....	79	20	1			

As I had never seen any signs of degeneration of the supernumeraries, the natural interpretation of their infrequency at La Jolla would seem to be either that they had originated here more recently, or that they had originally appeared in fewer individuals in this locality.

The behavior of supernumeraries in all cases where they have been shown to occur at once classes them with the heterochromosomes, and in *Metapodius* Wilson ('09) has shown that they have probably originated in an irregular second maturation mitosis in which both idiochromosomes went to one pole of the spindle instead of separating. He therefore regards the supernumeraries in *Metapodius* as duplicates of the smaller idiochromosome. In 1908 I suggested that there might be two varieties of *Diabrotica soror* and also of *D. 12-punctata*, one having only the odd heterochromosome and the other an unequal pair, and that hybridization might have given rise to the supernumeraries with their peculiar behavior, dividing sometimes in one sometimes in the other maturation mitosis. I have, however, been able to find no evidence in favor of this view. In 1910 I studied carefully the testes of many individuals where no supernumeraries were present, seeking some clue to the origin of these chromosomes.

As a rule the odd chromosome X appears near one pole of the spindle in the metaphase of the first maturation mitosis, but I had always noticed that occasionally X is in or near the equatorial plate, and in some individuals this is quite common. At La Jolla I found two spindles in which X was between the daughter plates in the anaphase, and stretched out lengthwise (Figs. 1 and 2). In one of these cases (Fig. 2) X was split so



FIGS. 1–5. Anaphases of first maturation mitosis, showing abnormal position and transverse division of *X*. (Mag. 1,500 for all figures.)

FIGS. 6*a* and *b*. Metaphase of first maturation mitosis, showing two supernumeraries (*s*) unequal in size.

FIGS. 7–10. Other stages from same testis showing behavior of *X*, and the two supernumeraries.

that it was certain that it was in a position such that it might divide transversely, but I was not able to find any cases of actual transverse division of *X*. Later at Mountain View I did find two anaphases where *X* appeared to have divided transversely and unequally (Figs. 3 and 5) and one in which *X* was caught in the cell plate between the daughter cells (Fig. 4). Now the supernumeraries are usually very uniform in size and certainly less than one half the sixe of *X*. I have one individual noted as having an unusually large supernumerary, about one-half as large as *X*, and a few cases where an unusually small one occurs. One of the latter cases is shown in '08, Pl. III., Figs. 76 to 78. From the evidence now at hand I should infer that the probable origin of the supernumeraries in the *Diabroticas* has been an occasional transverse division of *X* followed by a longitudinal division of the two parts. Evidently the transverse division has usually been an equal one, but that it may be unequal is shown by Figs. 3 and 5, and the rather rare occurrence of unusually large and unusually small supernumeraries. Figs. 6 to 10 are from a male captured at Mountain View, July 29, 1910. Here we have a large and a small supernumerary in the same individual. In the metaphase (6*a* and 6*b*) *X* and the two supernumeraries were all near one pole of the spindle, while in Figs. 8 and 9 the supernumeraries are at opposite poles and in Fig. 9, *X* is near the equatorial plate. In Fig. 10, *X* and both supernumeraries have gone undivided to one second spermatocyte. No cases of the division of either supernumerary in the first maturation mitosis were found in this individual.

In *Metapodius* Wilson found no somatic variations corresponding to the variation in the number of supernumeraries. In fact the insects with *X* alone, *X* and *Y*, or *X*, *Y* and 1 to 6 supernumeraries are described as indistinguishable. These species of *Diabrotica* are very variable in size, and in regard to size and fusion of the 12 black spots on the elytra, but as I showed in 1908 there is no significant correlation between these somatic variations and the presence or absence or number of supernumeraries ('08, Tables I. and II., and p. 465, text). In *Metapodius* the indications are that the chromosome *Y* is of no hereditary value. and the supernumeraries, as duplicates of *Y* would not be ex-

pected to affect the somatic characteristics of the insects. If, however, the supernumeraries of *Diabrotica* come originally from different regions of *X*, there would seem to be no reason why they should not bear functional genes for sex and other characters. The male always contains *X* so far as my experience goes (over 700 males), but one would suppose, if the supernumeraries are functional in heredity, that one *X* and a supernumerary might frequently determine the development of a female, and if so there should be males without *X*, but with a supernumerary in its place. It may, of course, be true that the abnormal division of *X* producing supernumeraries in itself indicates a degenerate or non-functional condition of that particular *X* chromosome, and therefore of its progeny—the resulting supernumeraries. This would fall in line with Schleip's ('11) suggestion in regard to the rejected *X* chromosome in the spermatogenesis of the hermaphrodite generation of *Angiostomum nigrovenosum*, that it had already become non-functional at an earlier stage, whence its later behavior. It is exceedingly desirable that the female sex cells of these *Diabroticas* should be studied, but I have not been able to get any favorable mitoses in the adults, or to secure larvæ or pupæ from the soil or roots of plants on which they live. Several attempts to breed them have given no results.

The testes of 12 males each having one supernumerary were studied from the point of view of the division of the supernumeraries in the first maturation mitosis. All anaphases and metaphases in each preparation were examined and all cases where it was possible to determine the position and behavior of the supernumerary recorded. In the metaphase the supernumerary was in the equatorial plate in 54.1 per cent. of 901 cases and out of the plate—nearer one pole of the spindle—in 45.9 per cent. Apparently the supernumeraries, when they divide, do so later than the bivalent chromosomes, so all anaphases were examined on this point. In 55.6 per cent. of the anaphases found in the 12 testes, the supernumerary was between the daughter plates, and in 44 per cent. it was dividing or divided. Here the 56.6 per cent. corresponds closely with the 54.1 per cent. in the equatorial plates in the metaphase, and the 44.4 per cent. outside of the daughter plates in anaphases comes

very near the 45.9 per cent. out of the equatorial plates in metaphase. The division of supernumeraries or their failure to divide in the first maturation mitosis seems to be a matter of chance, depending on their position in the spindle in the prophase and on the attachment of spindle fibers from one or from both poles of the spindle. In Fig. 11 both supernumeraries are connected by fibers with both poles, in Fig. 12 the *s*-chromosome is connected with both poles and is about to divide, and in Fig. 13 one *s*-chromosome is connected with both poles and will later divide, while the other will go undivided to the upper pole of the spindle and therefore to one second spermatocyte. The behavior of the other chromosomes indicates a more or less definite attachment point for the spindle fibers, near the middle of the chromo-

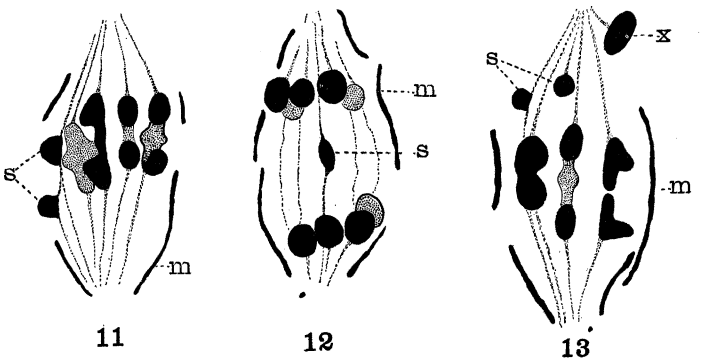


FIG. 11. Spindle showing two supernumeraries (*s*), each attached to spindle fibers from both poles. Mag. 2,000.

FIG. 12. Anaphase showing supernumerary (*s*) about to divide.

FIG. 13. Metaphase showing *X*, a supernumerary (*s*) attached to one spindle fiber, and another (*s*) attached to two. *m* = mitochondria.

some in both spermatogonial and spermatocyte mitosis (Figs. 11 and 13). The supernumeraries seem to be able to make connections with both poles in most cases if they are in or near the equatorial plate in late prophase stages.

SEX RATIOS.

The sex ratios in *Diabrotica soror* and *Diabrotica 12-punctata* have shown very peculiar variations. In studying the male germ cells of *D. soror* in 1907 I made no note of the number of females found in random collections, but in dissecting *D. 12-*

punctata in October, 1907, I found more than two males to one female,—in one lot 58 males to 25 females. In 1909 the number of males and females was noted for each lot dissected. Between July 10 and August 12, 107 males and 102 females were counted in random collections from two neighboring gardens, but it was noticed that the ratios in the two gardens were quite different. In garden *A* there were 58 males to 26 females; in garden *B*, 49 males to 76 females. A second lot from garden *A* collected between August 21 and September 15 gave 101 males to 24 females. The percentage of females in garden *A*, first lot, was 30.9, second lot 19.2, average 23.9, and for garden *B* 60.8.

At La Jolla in 1910 the ratios ran more evenly.

	♂		♀
La Jolla, June 17 and 18	25	:	16
National City, June 22	34	:	34
La Jolla, June 28	14	:	19
La Jolla, July 1	14	:	25
La Jolla, July 4	20	:	27
	107	:	121

At Mountain View again the ratios were peculiar. Five random collections in Garden *A* gave 100 males to 20 females, and two other later collections 76 males to 18 females. Only a few were collected from garden *B* giving 12 males to 6 females. In 1911 mixed lots from both gardens gave more males than females, 61 : 47. These were recorded incidentally while fixing a lot of testes for sections. By referring to the table on page 232, it will be seen that the number of supernumeraries runs about the same for the first 100 in 1909, about one half of which came from each garden (*A* 51 and *B* 49), and for the second 100, all of which came from garden *A*. It therefore seems unlikely that the supernumeraries have anything to do with the difference in sex ratios in the two gardens. The soil in garden *A* is harder in summer—more adobe in it—and less thoroughly cultivated than *B*. Two possibilities are suggested in this connection: (*a*) The males may be more successful in pupating and escaping from the hard soil than the females or (*b*) few of either sex may emerge from the hard soil in garden *A*, and the males may be better flyers and so come in larger numbers from other neighboring gardens. The latter is regarded as more probable.

The Bryn Mawr *Diabroticas* of 1907 were all collected on a large clump of golden rod in a pasture that had not been cultivated for many years, and they may have come out of the ground in the immediate neighborhood or from more recently cultivated fields near by.

These erratic sex ratios are probably merely another example of the interference of external conditions in what would otherwise be an equality of sexes, or in other words a shifting of normally equal sex ratios, or partial exclusion of one sex by peculiarities in the environment. The collections were all random in the sense that all the individuals that could be found were collected each time.

BRYN MAWR COLLEGE,
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